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(54) Title: SAPONIN ADJUVANTS IN COMBINATION WITH DNA VACCINATION			
(57) Abstract  Methods of immunizing a vertebrate, by inoculating the vertebrate with a nucleic acid vaccine in conjunction with a saponin adjuvant, such as the adjuvant QS-21, are disclosed. The saponin adjuvant can be administered within approximately 10 days, preferably within approximately 5 days, and more preferably within approximately 2 days, before the nucleic acid vaccine; contemporaneously with the vaccine; or within approximately 10 days, preferably within approximately 7 days, and more preferably within approximately 1 day, after the nucleic acid vaccine. Compositions comprising a nucleic acid vaccine and a saponin adjuvant are also disclosed.			

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## SAPONIN ADJUVANTS IN COMBINATION WITH DNA VACCINATION

RELATED APPLICATIONS

This application is a Continuation-in-part (CIP) application of U.S. Serial No. 08/848,310, filed April 30, 5 1997, which claims the benefit of U.S. Serial No. 60/043,823, filed April 14, 1997. The entire teachings of these applications are incorporated herein by reference.

GOVERNMENT SUPPORT

Work described herein was supported by Grant 5-25143 10 from the National Institutes of Health. The U.S. Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The delivery of antigen-encoding DNA for the elicitation of immune response, has become an enticing 15 possibility for vaccination against many diseases, such as influenza (Robinson, H.L. et al., *Vaccine* 11(9):957-960 (1993); Fynan, E.F. et al., *Proc. Natl. Acad. Sci. USA* 90:11478-11482 (1993); Fynan, E.F. et al., *DNA and Cell Biol.* 12(9):785-589 (1993); Ulmer, J.B. et al., *Science* 20 259:1745-1749 (1993); WO93/19183; and WO94/21797) hepatitis B (Davis, H.L. et al., *Hum. Mol. Gen.* 2(11):1847-11851 (1993)); and immunodeficiency viruses, such as human

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immunodeficiency virus (Lu, S. et al., *Virology* 209:147-154 (1995); Lu, S. et al., *J. Virol.* 70:3978-3991 (1996)). DNA immunization avoids the disadvantages that are usually associated with traditional vaccines, such as low immunogenicity, particularly for killed-organism-based vaccines in which conformational epitopes may have been distorted; the risk of infection from live attenuated vaccines; and expensive production, transportation and storage, particularly due to the need for refrigeration for recombinant subunit vaccines. Furthermore, in contrast with inactive or subunit vaccines, DNA vaccines are particularly effective in raising cytotoxic T-lymphocytes, which are important in clearing viral infections. Because of the continued demand for effective, low-cost vaccines, a need remains for means to enhance the antigenicity and efficacy of DNA immunization.

#### SUMMARY OF THE INVENTION

The current invention is drawn to methods of immunizing a vertebrate, by administering a nucleic acid vaccine, in conjunction with a saponin adjuvant, such as QS-21 (Aquila Biopharmaceuticals Inc., Cambridge, MA). The saponin adjuvant can be administered within approximately 10 days, preferably within approximately 5 days, and more preferably within approximately 2 days, of inoculation with the nucleic acid vaccine; within approximately 10 days, and preferably within approximately 7 days, and more preferably within approximately 1 day, after administration of the

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vaccine; within 24 hours of the nucleic acid vaccine; or contemporaneously with the nucleic acid vaccine. In one embodiment of the invention, the nucleic acid vaccine includes a polynucleotide encoding an antigen from human immunodeficiency virus-1 (HIV-1), such as envelope protein. In another embodiment of the invention, the nucleic acid vaccine includes a polynucleotide encoding an antigen from hepatitis B, such as core antigen. The invention is further drawn to compositions comprising a nucleic acid vaccine and a saponin adjuvant.

Timely administration of a saponin adjuvant in conjunction with a nucleic acid vaccine generates antibody titers and/or cytotoxic T lymphocyte (CTL) responses that are significantly higher than the antibody titers generated in the absence of the adjuvant. The methods of the invention thereby significantly improve the immune response to the nucleic acid vaccine, and thereby enhance efficacy of the vaccine, reduce the amount of nucleic acid vaccine necessary to induce a protective immune response, and improve immunogenicity of nucleic acid vaccines encoding weak antigens.

#### DETAILED DESCRIPTION OF THE FIGURES

Figure 1 is a graphic representation of levels of anti-gp120 antibodies over time in Balb/C mice inoculated with a nucleic acid vaccine encoding the gp120 form of envelope protein of Human Immunodeficiency Virus-1 (HIV-1). Open squares, nucleic acid vaccine encoding gp120 protein;

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filled circles, nucleic acid vaccine encoding gp120 protein + QS-21 administered 2 days before inoculation with the vaccine; filled squares, nucleic acid vaccine encoding gp120 protein + QS-21 administered 1 day after inoculation with the vaccine; Xs, vector DNA (negative control vaccine) + QS-21 administered 2 days before and 1 day after inoculation with the vaccine.

Figure 2 is a graphic representation of anti-gp120 IgG antibody titers in Balb/C mice inoculated with a nucleic acid vaccine encoding the gp120 protein of HIV-1. Treatment groups are described in Table I.

Figure 3 is a graphic representation of cytotoxic T lymphocyte (CTL) function induced in mice, measured using HIV-1 strain IIIB envelope (ENV) peptide-coated P815 cells as targets in a  $^{51}\text{Cr}$  release assay. E:T ratio, effector-target ratio. Filled circles, nucleic acid vaccine encoding gp120 protein; open squares, nucleic acid vaccine encoding gp120 protein; filled circles, nucleic acid vaccine encoding gp120 protein + QS-21 administered 2 days before inoculation with the vaccine; filled squares, nucleic acid vaccine encoding gp120 protein + QS-21 administered 1 day after inoculation with the vaccine; filled triangles, nucleic acid vaccine encoding gp120 protein + QS-21 administered 2 days before and 1 day after inoculation with the vaccine; +, vector DNA (negative control vaccine) + QS-21 administered 2 days before and 1 day after inoculation with the vaccine.

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Figure 4 is a graphic representation of anti-hepatitis B core antigen antibody titers in C57/BL6 mice inoculated with a nucleic acid vaccine encoding the core antigen of hepatitis B. Solid lines, group immunized with nucleic acid vaccine alone; hatched lines, group immunized with nucleic acid vaccine in conjunction with QS-21.

#### DETAILED DESCRIPTION OF THE INVENTION

The current invention pertains to the use of a saponin adjuvant in conjunction with DNA immunization, and to compositions comprising a saponin adjuvant and a nucleic acid vaccine. As described herein, Applicant has discovered that a saponin adjuvant, QS-21 (an acylated triterpene glycoside isolated from the bark of the South American tree *Quillaja saponaria* Molina, also referred to as Stimulon (Aquila Biopharmaceuticals Inc., Cambridge, MA)), when administered in conjunction with DNA immunization, boosts higher antibody and CTL responses. Mice inoculated with a nucleic acid vaccine comprising DNA encoding gp120 protein from human immunodeficiency virus-1 (HIV-1), in conjunction with QS-21, generated antibody titers to gp120 protein that were approximately 10-fold higher than mice inoculated with the same nucleic acid vaccine in the absence of QS-21. Furthermore, use of QS-21 in conjunction with the nucleic acid vaccine generated a significantly higher specific CTL response than use of the nucleic acid vaccine alone. Use of QS-21 in conjunction with a nucleic acid vaccine comprising DNA encoding core

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antigen from hepatitis B similarly generated significantly higher antibody titers.

As a result of this discovery, methods of enhancing the efficacy of DNA immunization, by administering a saponin adjuvant in conjunction with a nucleic acid vaccine, as well as compositions comprising a saponin adjuvant and a nucleic acid vaccine, are now available. The term, "DNA immunization," as used herein, refers to inoculation of a vertebrate, particularly a mammal, with a nucleic acid vaccine directed against a pathogenic agent, resulting in protection of the vertebrate against disease caused by the pathogenic agent. Representative vertebrates include mice, dogs, cats, chickens, sheep, goats, cows, horses, pigs, non-human primates, and humans. A "nucleic acid vaccine," as used herein, is a nucleic acid construct comprising a polynucleotide encoding a polypeptide antigen. The nucleic acid construct can also include transcriptional promoter elements; enhancer elements; splicing signals; termination and polyadenylation signals; and other nucleic acid sequences that may be present in an expression vector.

The polypeptide antigen encoded by the nucleic acid vaccine can be any antigen of a pathogenic agent (e.g., any antigen encoded by DNA or RNA of the pathogenic agent, or expressed by the pathogenic agent). For example, the polypeptide antigen can be core antigen from hepatitis B, or can be envelope protein of the human immunodeficiency virus-1 (HIV-1), such as the gp-120 form of envelope protein. Alternatively, the polypeptide antigen can be a



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polypeptide which, although not encoded by DNA or RNA of the pathogenic agent, or expressed by the pathogenic agent, has been determined to be useful in raising a protective immune response against disease caused by the pathogenic agent. The polypeptide antigen can also be a tumor antigen. The polypeptide antigen may or may not be structural components of the pathogenic agent; it can undergo normal host cell modifications such as glycosylation, myristoylation or phosphorylation. The polypeptide antigen can be designed to undergo intracellular, extracellular or cell-surface expression.

Potential pathogenic agents for which the invention is useful include viruses, bacteria, fungi, parasites and other pathogens. Representative viruses include herpesviruses, orthomyxoviruses, rhinoviruses, picornaviruses, adenoviruses, paramyxoviruses, coronaviruses, rhabdoviruses, togaviruses, flaviviruses, bunyaviruses, rubella virus, reovirus, hepadna viruses, and retroviruses including human immunodeficiency virus (HIV). Representative bacteria include mycobacteria, spirochetes, rickettsias, chlamydia and mycoplasma. Representative fungi include yeasts and molds. It is to be understood that the pathogenic agents listed above are representative of the numerous pathogenic agents for which protection against disease can be generated according to the methods and through the use of compositions described herein.

"Protection against disease caused by the pathogenic agent" refers to generation of an immune response in the

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vertebrate, the immune response being protective (partially or totally) against manifestations of the disease caused by the pathogenic agent. A vertebrate that is protected against disease caused by the pathogenic agent may be  
5 infected with the pathogenic agent, but to a lesser degree than would occur without immunization; may be infected with the pathogenic agent, but does not exhibit disease symptoms; or may be infected with the pathogenic agent, but exhibits fewer disease symptoms than would occur without  
10 immunization. Alternatively, the vertebrate that is protected against disease caused by the pathogenic agent may not become infected with the pathogenic agent at all, despite exposure to the pathogenic agent.

The nucleic acid vaccine can be produced by standard  
15 methods. For example, using known methods, a nucleic acid encoding the polypeptide antigen of interest can be inserted into an expression vector to construct a nucleic acid vaccine (see Maniatis et al., *Molecular Cloning, A Laboratory Manual*, 2nd edition, Cold Spring Harbor  
20 Laboratory Press (1989)).

The individual vertebrate is inoculated with the nucleic acid vaccine (i.e., the nucleic acid vaccine is administered), using standard methods. The vertebrate can be inoculated subcutaneously, intravenously,  
25 intraperitoneally, intradermally, intramuscularly, topically, orally, rectally, nasally, buccally, vaginally, by inhalation spray, or via an implanted reservoir in dosage formulations containing conventional non-toxic,

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physiologically acceptable carriers or vehicles.

Alternatively, in a preferred embodiment, the vertebrate is inoculated with the nucleic acid vaccine through the use of a particle acceleration instrument (a "gene gun"). The form in which it is administered (e.g., capsule, tablet, solution, emulsion) will depend in part on the route by which it is administered. For example, for mucosal administration, via nose drops, inhalants or suppositories can be used. For administration with a particle acceleration instrument, the nucleic acid vaccine can be coated on to appropriate particles, such as gold beads.

The nucleic acid vaccine is administered in conjunction with the saponin adjuvant. The term, "saponin," as used herein, refers to a compound extracted from the bark of the South American tree, *Quillaja saponaria* Molina. Numerous saponins have been extensively described (see, for example, U.S. patent 5,057,540; Kensil, C.R. et al. (1991), *J. Immunol.* 146:431-437). In a preferred embodiment of the invention, the adjuvant is the saponin, QS-21 (Stimulon; Aquila Biopharmaceuticals Inc., Worcester, MA; see, for example, Kensil, C.R. et al., "Structure/Function Studies on QS-21, a Unique Immunological Adjuvant from *Quillaja saponaria*," pp. 165-172, Saponins Used in Traditional and Modern Medicine, Waller and Yamasaki, Plenum Press, New York, 1996; Kensil, C.R. et al., "Structural and Immunological Characterization of the Vaccine Adjuvant QS-21," pp. 525-541, Vaccine Design: The Subunit and Adjuvant Approach, Powell and

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Newman, Plenum Press, New York, 1995. In another preferred embodiment, the saponin is a derivative of QS-21 (see, for example, Soltysik, S. et al., *Vaccine* 13(15):1403-1410 (1995)).

5       The adjuvant is administered in a sufficient amount, which is that amount that is sufficient to generate an enhanced immune response to the nucleic acid vaccine. For example, a sufficient amount can be determined as described by Kensil et al. (Kensil, C.R. et al., *Vaccine Research* 10 2(4):273-2281 (1993); see also Newman (Newman, M.J. et al., *AIDS Research and Human Retroviruses* 8(8):1413-1418 (1992); Kensil, C.R. et al., "Structure/Function Studies on QS-21, a Unique Immunological Adjuvant from *Quillaja saponaria*," pp. 165-172, Saponins Used in Traditional and Modern 15 Medicine, Waller and Yamasaki, Plenum Press, New York, 1996; Kensil, C.R. et al., "Structural and Immunological Characterization of the Vaccine Adjuvant QS-21," pp. 525-541, Vaccine Design: The Subunit and Adjuvant Approach, Powell and Newman, Plenum Press, New York, 1995. The 20 entire teachings of all of the references cited are incorporated herein by reference in their entirety).

      The saponin adjuvant can be administered prior to (e.g., 1 or more days before) inoculation with the nucleic acid vaccine; concurrently with (e.g., within 24 hours of) 25 inoculation with the nucleic acid vaccine; contemporaneously (simultaneously) with the nucleic acid vaccine (e.g., the saponin adjuvant is mixed with the nucleic acid vaccine, and the mixture is administered to

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the vertebrate); or after (e.g., 1 or more days after) inoculation with the nucleic acid vaccine. The saponin adjuvant can also be administered at more than one time (e.g., prior to inoculation with the nucleic acid vaccine and also after inoculation with the nucleic acid vaccine). As used herein, the term "in conjunction with" encompasses any time period, including those specifically described herein and combinations of the time periods specifically described herein, during which the adjuvant can be administered so as to generate an enhanced immune response to the nucleic acid vaccine (e.g., an increased antibody titer to the antigen encoded by the nucleic acid vaccine, or an increased antibody titer to the pathogenic agent) in comparison to administration of the nucleic acid vaccine without the adjuvant. In a preferred embodiment, the saponin adjuvant is administered within approximately 10 days before inoculation with the nucleic acid vaccine; in a more preferred embodiment, the saponin adjuvant is administered within approximately 5 days before inoculation with the nucleic acid vaccine; in an even more preferred embodiment, the saponin adjuvant is administered within approximately 2 days before inoculation with the nucleic acid vaccine. In another preferred embodiment, the saponin adjuvant is administered within approximately 10 days after inoculation with the nucleic acid vaccine; in another, more preferred embodiment, the saponin adjuvant is administered within approximately 7 days after inoculation with the nucleic acid vaccine; in an even more preferred embodiment,

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the saponin adjuvant is administered within approximately 1 day after inoculation with the nucleic acid vaccine. In yet another preferred embodiment, the saponin adjuvant is administered contemporaneously.

- 5       The adjuvant and the nucleic acid vaccine can be administered at approximately the same location on the vertebrate: for example, both the adjuvant and the nucleic acid vaccine are administered at a marked site on a limb or on the abdomen of the vertebrate.
- 10       The invention is further illustrated by the following Examples.

EXAMPLE 1:       BOOSTING OF ANTIBODY TITER TO gp120 WITH QS-  
21 ADJUVANT

- 15       A first set of experiments was performed with Balb/C mice to evaluate antibody response generated by inoculation with a nucleic acid construct that encodes gp120 protein from human immunodeficiency virus-1 (HIV-1).

Five groups of mice, with five mice per group, were inoculated as follows:

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Table I Inoculation regimens

Group	Inoculation Regimen
A	gp120 DNA inoculation alone
B	gp120 DNA inoculation + QS-21 given 2 days prior to DNA inoculation
5 C	GP120 DNA inoculation + QS-21 given 1 day after DNA inoculation
D	gp120 DNA inoculation + QS-21 given 2 days prior and 1 day after DNA inoculation
E	Vector DNA (control) + QS-21 given 2 days prior and 1 day after DNA inoculation

For each immunization, each mouse received 6 gene gun shots of 1  $\mu$ g DNA each. QS-21 was injected subcutaneously by using a 1 cc syringe. The sites were marked and the DNA inoculations were given to the same spot. Each mouse received a total of 20  $\mu$ g of QS-21 (100  $\mu$ g/ml in PBS) per immunization.

Sera were collected at the time points shown in Figure 1, and an anti-gp120 enzyme-linked immunosorbent assay (ELISA) was performed, using recombinant gp120 as the coating antigen. Results are shown in Figure 1. In addition, titers of anti-gp120 IgG antibody were calculated at bleed 7 (14.5 weeks from the first immunization) (Figure 2).

The results demonstrated that the adjuvant QS-21 boosted anti-gp120 antibody responses: antibody responses were increased approximately 10-fold with the adjuvant. QS-21 was more effective in boosting a higher anti-gp120 antibody response at the priming immunization. Higher CTL

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responses were achieved even when the adjuvant was administered prior to, or after, inoculation with the DNA vaccine.

Experiments were also performed to measure the activity of cytotoxic T-lymphocyte (CTL) cells generated in the mice after inoculation with the DNA vaccine. The assay, a  $^{51}\text{Cr}$  release assay using HIV-1 IIIB ENV peptide-coated P815 cells as targets, was performed essentially as described by Lu et al. (*Virology* 209:147-154 (1995), the teachings of which are incorporated herein in their entirety).

The results, shown in Figure 3, demonstrate that use of the saponin adjuvant, QS-21, greatly enhanced the percent of specific lysis in the CTL assay.

15   EXAMPLE 2:        REAFFIRMATION OF THE BOOSTING OF ANTIBODY  
                          TITER TO gp120 WITH QS-21 ADJUVANT

A second set of experiments was performed with Balb/C mice, with an expanded time frame of administration of the QS-21 adjuvant, to evaluate antibody response generated by inoculation with the nucleic acid construct encoding HIV-1 gp120 protein.

Nine groups of mice, with five mice per group, were inoculated as follows:



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Table II Inoculation regimens

Group	Inoculation Regimen
A	gp120 DNA inoculation alone
B	gp120 DNA inoculation + QS-21 given 10 days prior to DNA inoculation
5 C	gp120 DNA inoculation + QS-21 given 5 days prior to DNA inoculation
D	gp120 DNA inoculation + QS-21 given 2 days prior to DNA inoculation
E	gp120 DNA inoculation + QS-21 given the same day
F	gp120 DNA inoculation + QS-21 given 1 day after DNA inoculation
G	gp120 DNA inoculation + QS-21 given 7 days after DNA inoculation
10 H	Vector DNA (control) given 2 days prior and 1 day after DNA inoculation
I	Vector DNA (control) + QS-21 given 2 days prior and 1 day after DNA inoculation

For each immunization, each mouse received 6 gene gun shots of 1  $\mu$ g DNA each. QS-21 was injected subcutaneously by using a 1 cc syringe. The sites were marked and the DNA inoculations were given to the same spot. Each mouse received a total of 20  $\mu$ g of QS-21 (100  $\mu$ g/ml in PBS) per immunization. Mice were vaccinated three times, at one-month intervals.

Sera were collected at several time points, and an anti-gp120 enzyme-linked immunosorbent assay (ELISA) was performed to determine the end point titers for anti-gp120 antibodies at two weeks after the third immunization.

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Recombinant gp120 was used as the coating antigen. Results are shown in Table III.

Table III Gene Gun Inoculation Mouse anti-gp120 Peak  
Antibody Titers

5	Group	Inoculation Regimen	End-point Titration
	A	gp120 DNA inoculation alone	1:4050
	B	gp120 DNA inoculation + QS-21 given 10 days prior to DNA inoculation	1:4050
	C	gp120 DNA inoculation + QS-21 given 5 days prior to DNA inoculation	1:8000
	D	gp120 DNA inoculation + QS-21 given 2 days prior to DNA inoculation	1:12150
10	E	gp120 DNA inoculation + QS-21 given the same day	1:36450
	F	gp120 DNA inoculation + QS-21 given 1 day after DNA inoculation	1:36450
	G	gp120 DNA inoculation + QS-21 given 7 days after DNA inoculation	1:12150
	H	Vector DNA (control) given 2 days prior and 1 day after DNA inoculation	<1:150
	I	Vector DNA (control) + QS-21 given 2 days prior and 1 day after DNA inoculation	<1:150

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These results reaffirmed that the adjuvant QS-21 boosted anti-gp120 antibody responses. Administration of QS-21 on the same day, or one day after, the DNA vaccine appeared to be the most effective in raising a high antibody response  
5 in this second set of experiments.

EXAMPLE 3: BOOSTING OF ANTIBODY TITER TO HEPATITIS B  
CORE ANTIGEN WITH QS-21 ADJUVANT

C57/BL6 mice (five per group) were immunized using a gene gun with a DNA vaccine expressing hepatitis B core  
10 antigen (HBcDNA) alone; with the DNA vaccine in conjunction with QS-21; or with vector alone (control). For each immunization, each mouse received 6 gene gun shots of 1  $\mu$ g DNA each. QS-21 was injected subcutaneously by using a 1 cc syringe. The sites were marked and DNA inoculations  
15 were given to the same spot. Each mouse received a total of 20  $\mu$ g of QS-21 (100  $\mu$ g/ml in PBS) per immunization. Mice were vaccinated three times, at one-month intervals. Sera were collected at several time points, and an ELISA was performed to determine the end-point titers of anti-  
20 hepatitis B core antigen antibodies. Results, shown in Figure 4, indicated that after two immunizations (indicated by arrows), the animals receiving QS-21 in conjunction with the DNA vaccine had antibody titer at the same level as those animals having 3 immunizations of DNA vaccine alone  
25 (without QS-21).

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EQUIVALENTS

Those skilled in the art will be able to recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific  
5 embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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## CLAIMS

What is claimed is:

1. A method of immunizing a vertebrate, comprising  
administering to the vertebrate a nucleic acid vaccine  
5 in conjunction with a saponin adjuvant.
2. The method of Claim 1, wherein the saponin adjuvant is  
QS-21.
3. The method of Claim 1, wherein the saponin adjuvant is  
administered within approximately 10 days before the  
10 nucleic acid vaccine.
4. The method of Claim 3, wherein the saponin adjuvant is  
administered within approximately 5 days before the  
nucleic acid vaccine.
5. The method of Claim 3, wherein the saponin adjuvant is  
15 administered within approximately 2 days before the  
nucleic acid vaccine.
6. The method of Claim 1, wherein the saponin adjuvant is  
administered within approximately 10 days after the  
vaccine.

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7. The method of Claim 6, wherein the saponin adjuvant is administered within approximately 7 days after the vaccine.
8. The method of Claim 6, wherein the saponin adjuvant is administered within approximately 1 day after the vaccine.
9. The method of Claim 1, wherein the saponin adjuvant is administered within 24 hours of the nucleic acid vaccine.
10. The method of Claim 1, wherein the saponin adjuvant is administered contemporaneously with the nucleic acid vaccine.
11. The method of Claim 1, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of the human immunodeficiency virus-1.
12. The method of Claim 9, wherein the antigen is envelope protein.
13. The method of Claim 1, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of hepatitis B.

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14. The method of Claim 13, wherein the antigen is core antigen.
15. An improved method of immunizing a vertebrate, in which the vertebrate is inoculated with a nucleic acid vaccine, wherein the improvement comprises administering a saponin adjuvant in conjunction with the nucleic acid vaccine.
16. A composition comprising a saponin adjuvant and a nucleic acid vaccine.
- 10 17. The composition of Claim 16, wherein the saponin adjuvant is QS-21.
18. The composition of Claim 17, wherein the nucleic acid vaccine comprises a polypeptide encoding an antigen of the human immunodeficiency virus.
- 15 19. The composition of Claim 18, wherein the antigen is envelope protein.
20. The composition of Claim 17, wherein the nucleic acid vaccine comprises a polypeptide encoding an antigen of hepatitis B.
- 20 21. The composition of Claim 20, wherein the antigen is core antigen.

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22. Products containing a nucleic acid vaccine and a saponin adjuvant as a combined preparation for simultaneous, separate or sequential use in immunizing a vertebrate.
- 5 23. The products of Claim 22, wherein the saponin adjuvant is QS-21.
24. The products of Claim 22 or Claim 23, wherein the saponin adjuvant is administered within approximately 10 days before the nucleic acid vaccine.
- 10 25. The products of Claim 24, wherein the saponin adjuvant is administered within approximately 5 days before the nucleic acid vaccine.
26. The products of Claim 25, wherein the saponin adjuvant is administered within approximately 2 days before the  
15 nucleic acid vaccine.
27. The products of Claim 22 or Claim 23, wherein the saponin adjuvant is administered within approximately 10 days after the nucleic acid vaccine.
28. The products of Claim 27, wherein the saponin adjuvant  
20 is administered within approximately 7 days after the nucleic acid vaccine.



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29. The products of Claim 28, wherein the saponin adjuvant is administered within approximately 1 day after the nucleic acid vaccine.
30. The products of Claim 22 or Claim 23, wherein the saponin adjuvant is administered contemporaneously with the nucleic acid vaccine.
31. The products of any one of Claims 22 to 30, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of human immunodeficiency virus-1.
32. The products of Claim 31, wherein the antigen is envelope protein.
33. The products of any one of Claims 22 to 30, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of hepatitis B.
34. The products of Claim 33, wherein the antigen is core antigen.
35. Use of products for the manufacture of a medicament for immunization, the products comprising a nucleic acid vaccine and a saponin adjuvant as a combined preparation for simultaneous, separate or sequential use.

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36. The use of Claim 35, wherein the saponin adjuvant is QS-21.
37. The use of Claim 35 or Claim 36, wherein the saponin adjuvant is administered within approximately 10 days before the nucleic acid vaccine.
38. The use of Claim 37, wherein the saponin adjuvant is administered within approximately 5 days before the nucleic acid vaccine.
39. The use of Claim 38, wherein the saponin adjuvant is administered within approximately 2 days before the nucleic acid vaccine.
40. The use of Claim 35 or Claim 36, wherein the saponin adjuvant is administered within approximately 10 days after the nucleic acid vaccine.
41. The use of Claim 40, wherein the saponin adjuvant is administered within approximately 7 days after the nucleic acid vaccine.
42. The use of Claim 41, wherein the saponin adjuvant is administered within approximately 1 day after the nucleic acid vaccine.

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43. The use of Claim 35 or Claim 36, wherein the saponin adjuvant is administered contemporaneously with the nucleic acid vaccine.
- 5 44. The use of any one of Claims 35 to 43, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of human immunodeficiency virus-1.
45. The use of Claim 44, wherein the antigen is envelope protein.
- 10 46. The use of any one of Claims 35 to 43, wherein the nucleic acid vaccine comprises a polynucleotide encoding an antigen of hepatitis B.
47. The use of Claim 46, wherein the antigen is core antigen.

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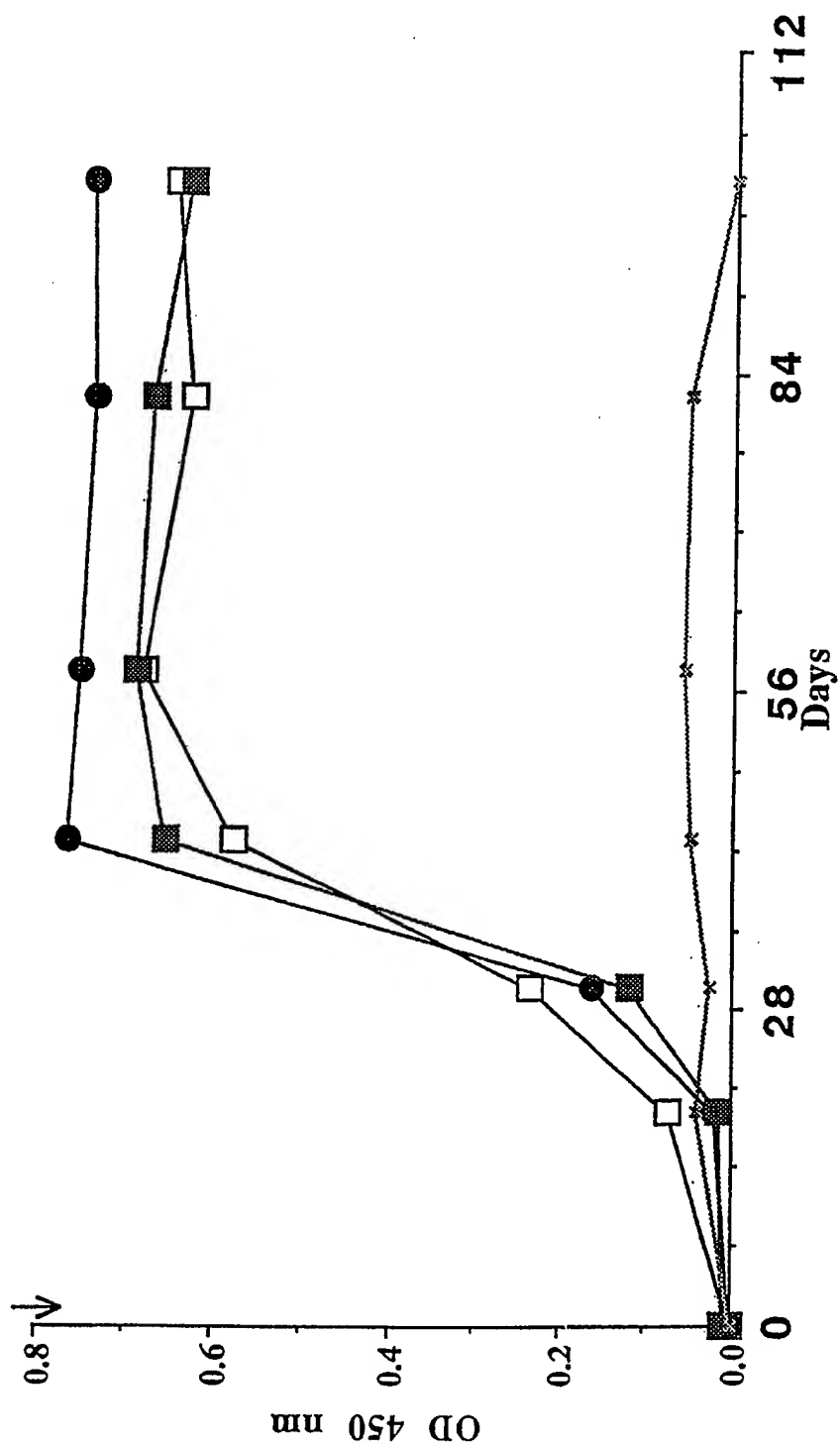


FIGURE 1

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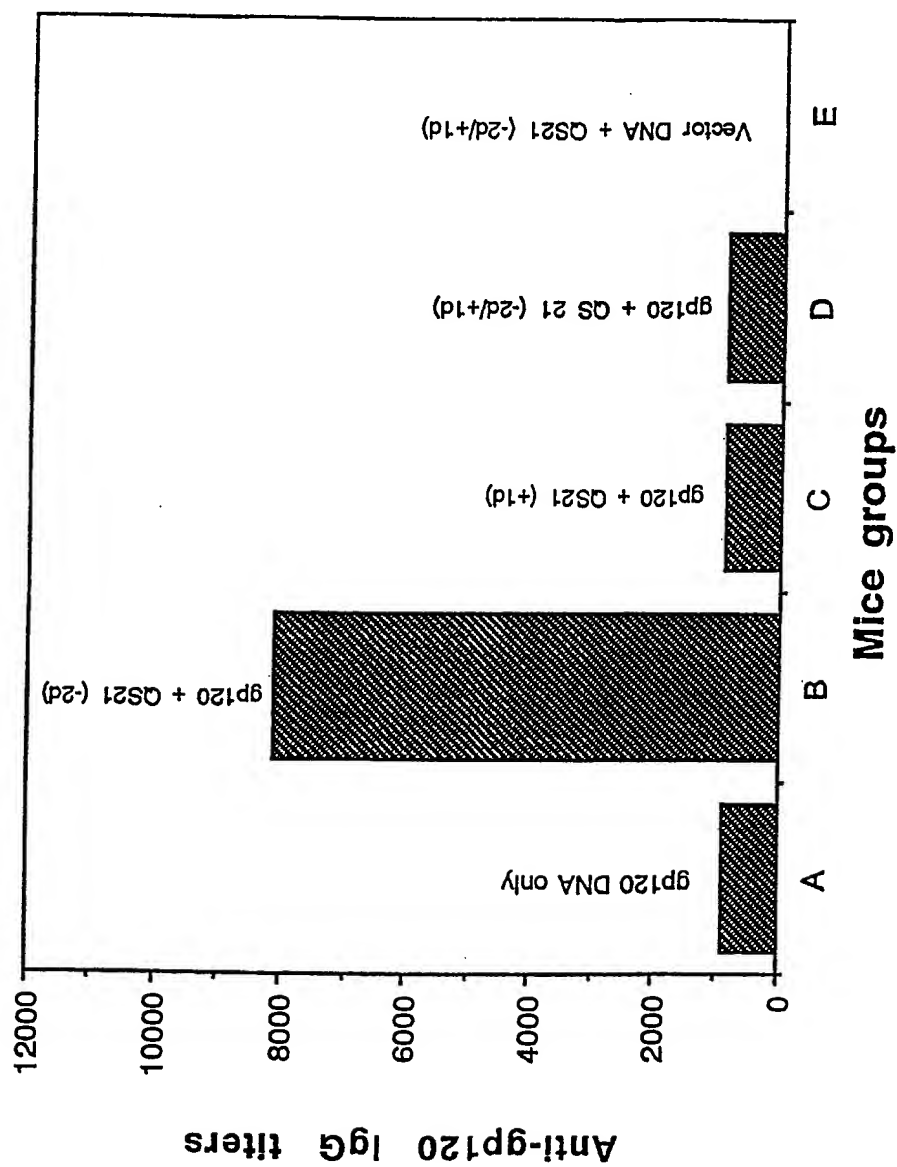


FIGURE 2

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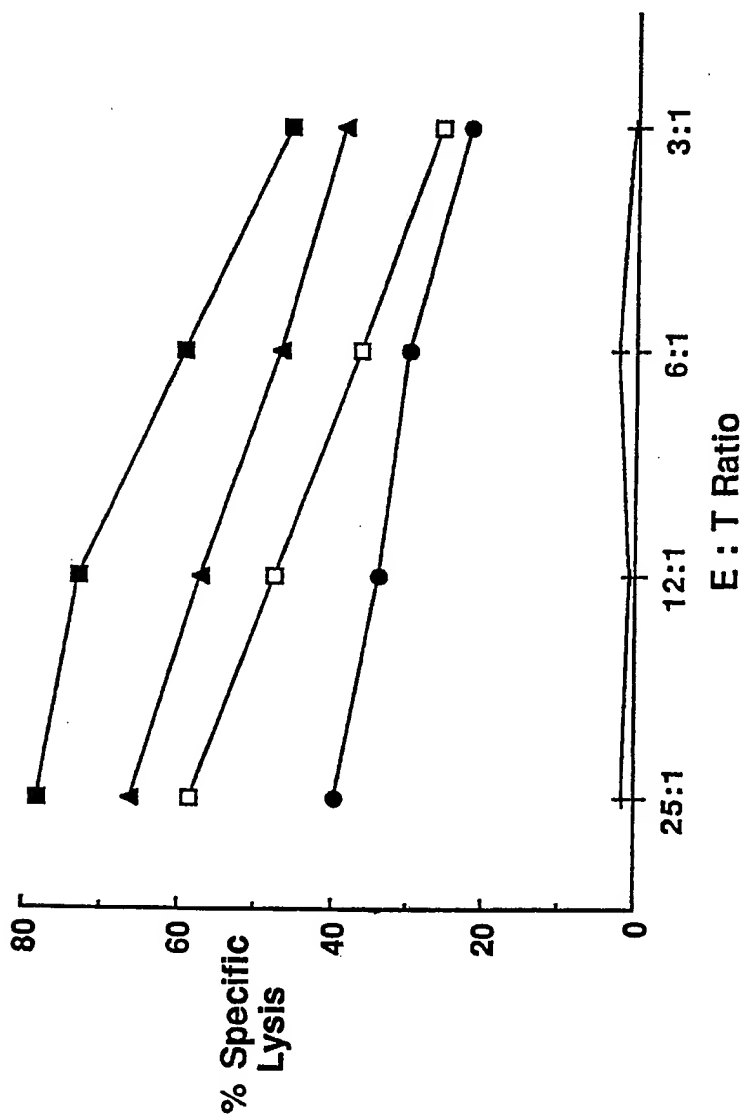


FIGURE 3

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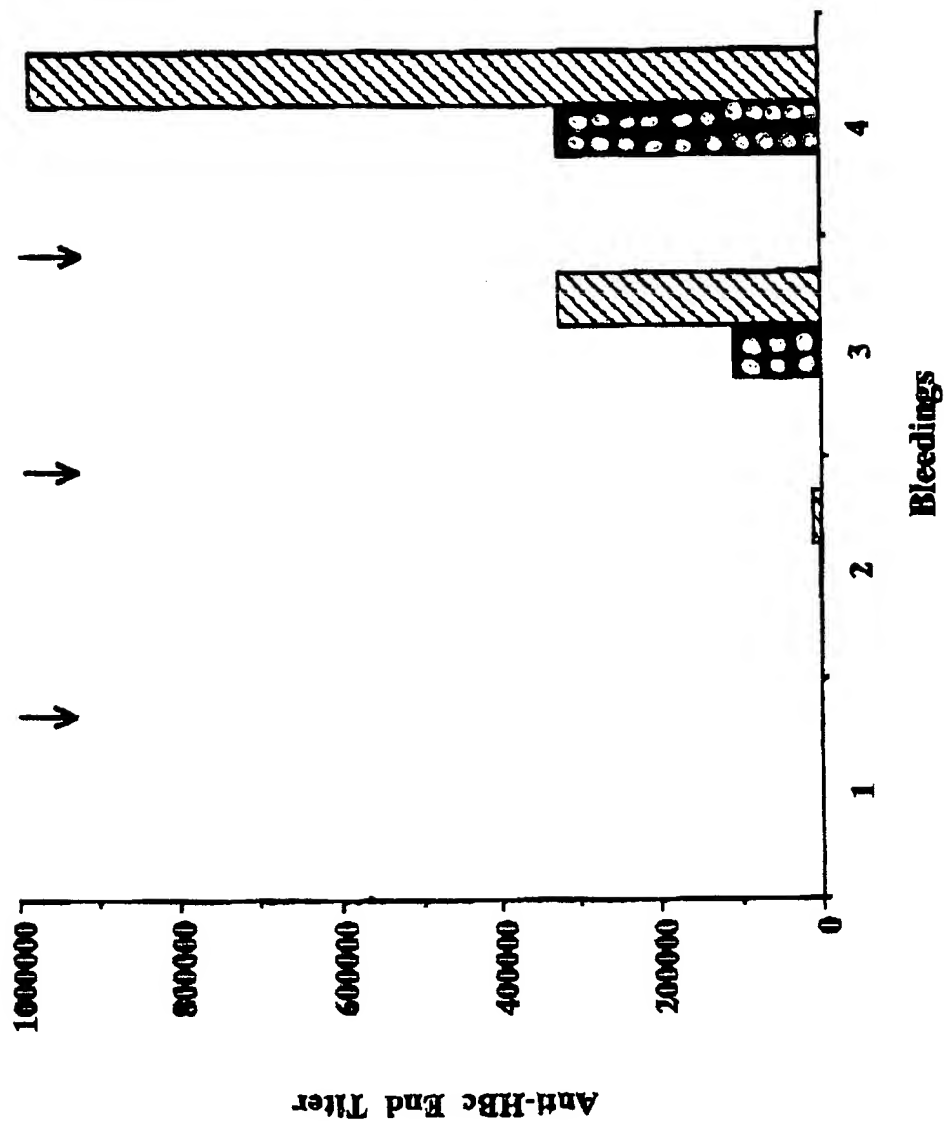


FIGURE 4

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/07218

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K39/39 //(A61K39/39, 39:21), (A61K39/39, 39:29)

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 04548 A (JENNER TECHNOLOGIES) 16 February 1995  see claims 1,5 ---	1-10, 15-17, 22-30, 35-43
X	WO 95 26718 A (APOLLON INC.) 12 October 1995 see claims 1-38 ---	1-47
X	WO 96 03998 A (UNIVERSITY OF SAKATCHEWAN) 15 February 1996 see page 12 --- -/--	1-47



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"A" document member of the same patent family

Date of the actual completion of the international search

29 July 1998

Date of mailing of the international search report

12/08/1998

Name and mailing address of the ISA

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Moreau, J



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/07218

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category <sup>2</sup>	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>DATABASE AIDSLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US WANG S. ET AL.: "QS-21 is effective in boosting anti-HIV-1 env antibody response induced by DNA immunization" XP002073000 see abstract &amp; CONF ADV AIDS VACCINE DEV, 1997, page 120</p> <p>-----</p>	1-47

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/07218

**Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  

Although claims 1-15  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 98/07218

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504548 A	16-02-1995	AU 686660 B	12-02-1998
		AU 7631294 A	28-02-1995
		EP 0721345 A	17-07-1996
		JP 9504000 T	22-04-1997
WO 9526718 A	12-10-1995	US 5739118 A	14-04-1998
		AU 2236695 A	23-10-1995
		CA 2186913 A	12-10-1995
		EP 0772437 A	14-05-1997
		JP 9511146 T	11-11-1997
WO 9603998 A	15-02-1996	US 5597807 A	28-01-1997
		US 5688772 A	18-11-1997
		AU 3338495 A	04-03-1996
		CA 2196082 A	15-02-1996
		EP 0773786 A	21-05-1997
		JP 10503495 T	31-03-1998